

STUDIES OF EVALUATION OF AN ENZYME PROCESS FOR UNHAIRING CATTLEHIDES*

ABSTRACT

An unhairing process using microbial enzymes was compared in matched-side experiments with the conventional lime-sulfide process in the production of side upper and sole leathers, with only a minimum modification of the conventional tanning processes. Leathers produced from stock unhaired with enzymes had most of the characteristics of leathers produced from stock unhaired conventionally. Matched sides of leather produced from enzyme versus lime-sulfide-unhaired stock were compared as to tannery sorters' evaluation, chemical analysis, and physical tests. The main difference between the side leather from the two unhairing systems was in temper: that from the enzyme-unhaired stock was one grade firmer. Also, the grain was somewhat coarser and the break somewhat looser. The sole leather from the stock unhaired with enzymes was not as uniform in color as the control lot, and there was some evidence of drawn grain; otherwise it was quite similar.



INTRODUCTION

The literature, including patents in the field of enzyme unhairing up to 1952, has been reviewed by Green (1). Merrill (2) has given a rather comprehensive review on hair loosening by enzymes in which he discussed animal proteases, which were first used for unhairing, the enzymes of vegetable origin, and those secreted by molds and bacteria, which were later investi-

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†A laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

gated. Although he gave no indication of the use of enzymes for unhairing on a commercial scale, it is well known that the Rohm & Haas Company's Arazyme* process (3) was successfully used by several goatskin tanners. An alkaline pretreatment and neutralization of the skins were necessary. With the discovery and ready availability of sodium sulfhydrylate this process was discontinued. As far as is known, Wallerstein's "Korofor"* process is still used by one kid tanner for one line of leather.

Studies at the Eastern Utilization Research and Development Division of the U. S. Department of Agriculture (4-6) have given information on the use of enzymes for unhairing cattlehides. A new approach that did not require alkaline pretreatment was taken. It was found (4) in a survey of some 40 enzyme preparations that several possessed rather marked activity for loosening hair. A bacterial protease designated H. T. Concentrate* was used in a test in which whole hides were treated and unhaired on conventional unhairing machines in a tannery. Some of the problems encountered in using the present tannery equipment and alterations in subsequent tanning procedures were presented. It was indicated that additional study was necessary to produce higher-quality leather before the enzyme unhairing system could be used commercially. Grimm (7) discussed the unhairing action of fungal, bacterial, and animal proteases and described a process in which powdered preparations are applied to the flesh side of green or soaked skins. He stated that it is possible to dewool sheepskins as well as unhair goatskins and cattlehides by the exclusive use of proteolytic enzymes from fungi and the required activators and that this has been carried out continuously in many tanneries (presumably in Germany) since 1949.

Lindroth (8) was able to unhair cattlehides in a pancreatic enzyme system with added sodium chloride. The resultant leather had a drawn grain and fine hair and was not as thick as that from matched samples unhaired by the conventional lime-sulfide process.

While the evidence seems clear that it is possible to remove the hair from animal hides by the use of enzymes without alkaline pretreatment, it is also true that problems had been encountered in making merchantable leather from enzyme-unhaired hides. The current work was done to obtain information necessary to convert laboratory results to tannery practice and to make commercially acceptable leather from enzyme-unhaired stock.

EXPERIMENTAL

Three enzyme preparations were used in laboratory tests, namely, a bacterial protease, H. T. Proteolytic*, from Miles Chemical Company, Clifton, New Jersey; a bacterial protease, L306, from the Pabst Laboratories,

*Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Milwaukee, Wisconsin; and a streptomyces protease, keratinase (now called M-ZYME®), from Merck & Co., Inc., Rahway, New Jersey.

Variables tested include concentration of the enzymes, temperature, pH, time, and agitation versus no agitation. Borax was used as a buffer in the enzyme baths, and 5% sodium chloride was added to increase the firmness of the unhaired hides.

Materials tested as soak assists on salt-cured hides for the enzyme treatment included sodium chloride in concentrations of 3% to 10% solutions, sodium tetrasulfide at concentrations of 0.2% to 0.8% based on the stock weight, and sodium hydroxide at concentrations of 0.2% to 0.5% also on the stock-weight basis.

Pickling conditions were studied for stock that was to be chrome-tanned. Salt concentration was varied from 6% to 12% and sulfuric acid concentration from 1.0% to 1.5% at a float of 1:1. The effect of treatment with salt for a few hours prior to addition of the acid was also investigated. No attempt was made to modify the tanning, retanning, or fatliquoring steps of the process.

Stock that was to be vegetable-tanned for sole leather was not given any further treatment after it was unhaired.

Pilot-Scale Tests

Pilot-scale tests using matched sides from five hides were run in side leather and sole leather tanneries. Information obtained from these tests was used in setting up plant-scale tests. M-ZYME® was selected for use in the plant-scale tests on the basis of its performance and ready availability.

Plant-Scale Tests

Side Upper Leather

Materials and procedures

Hides.—Salt-cured hides were sided, and cross-matched mates were used for enzyme unhairing tests and for lime-sulfide-unhaired controls.

Soak.—The sides were soaked for 22 hr. in 0.125% solution of sodium hydroxide at a 4:1 float. The sides were drummed for 5 min. at the beginning of each of the first 3 hr. At the end of 3 hr. the pH was 11.4, and the temperature was 71°F. At the end of 22 hr. the pH was 10.9, and the temperature was 72°F.

Wash.—The sides were washed in four changes of water over a 75-min. period. The temperature was raised from 70°F. to 95°F. The pH dropped from 10.3 to 9.0.

Enzyme treatment.—The washed sides were placed in a paddle vat containing

M-ZYME®.....	13.9 lb. (190 K units/ml.*)
NaCl.....	110 lb. (5% on solution basis)
Borax.....	15.4 lb. (0.7% on solution basis)
Water at 85°F.....	400%

The paddle was run 10 min. per hr. for the first 3 hr. and then held stationary for 45 hr. The temperature was raised to 96°F. after 22 hr. It had dropped to 84°F. at the end of 48 hr. The pH at the start was 8.8, and at the end it was 8.3.

Unhairing.—The sides were unhaired and scudded on the regular tannery equipment, then washed for 1 hr. at 70°F. without paddling and put in 12% sodium chloride for 5 hr.

Pickling and tanning.—After being held in a solution of 12% salt (15% in the 26-side test) for 5 hr. the sides were placed in the production mill with the regular stock which had been bated and washed but not pickled. The dynamic, pickle-tan process was then followed.

The control sides were processed by the regular tannery procedure which included unhairing with lime-sulfide for 2 days.

Evaluation of leather.—The stock was examined at several stages during the processing by tannery personnel: after unhairing, after tannage in the blue, in the crust, after burnishing, and after finishing. Chemical analyses of the leather were made for moisture, grease, hide substance, ash, chrome, and pH (9). The following physical properties were also determined: tensile strength, elongation at break, grain crack, stitch tear (9), and compressibility (temper) (10). All characteristics of the experimental leather were compared with the matched sides that had been processed by the regular tannery procedures. Where applicable the data were analyzed statistically. Thin cross sections were made of leather samples for the observation of fiber structure and dye penetration and for incineration to show chrome deposition (11). The shrinkage temperature of the leathers was also determined (9).

Results and discussion.—This tannery uses a five-point system of grading to evaluate the unhairing. Good unhairing was accomplished in both runs when the enzyme system was used. Thirty-one of the 50 sides had less hair than those unhaired conventionally, and only 8 had more. The tannery sorters' evaluation of the leather in the various stages of processing is given in Table I. In the blue the lime-unhaired stock was slightly thicker than the enzyme-unhaired stock; there was no difference in footage yield. The crust evaluation before sammying and staking showed the enzyme stock

*Keratinase activity per ml.

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to be firmer than the lime-sulfide stock. In the first run, five sides of enzyme stock had somewhat more hair remaining than there was on the lime-sulfide stock; in the second, 11 of the conventionally unhaired sides had more hair than there was on the enzyme stock. After burnishing, the temper was still somewhat firmer, the break was equal or somewhat looser, and the grain was somewhat coarser in the majority of the sides of the enzyme-unhaired stock.

TABLE I
TANNERY SORTERS' EVALUATION OF SIDE
UPPER LEATHER IN VARIOUS STAGES OF PROCESSING

Raw Stock				
	First Run (24 Sides)	Second Run (26 sides)		
Wt. of Hides, lb.	550	610		
<u>Characteristic</u>	<u>Test</u>	<u>Control</u>	<u>Test</u>	<u>Control</u>
In the Blue				
Pre-split thickness, avg., oz.				
Butt	10.00	10.48	10.72	11.08
Center	8.52	9.06	8.67	9.40
Front pocket	—	—	3.14	3.51
Measurement, sq. ft., avg.	—	—	18.40	18.21
Hair present*	Less	12	19	
	Equal	5	6	
	More	7	1	
In the Crust				
Temper* before sammying and staking	Firmer	24	26	
	Equal	0	0	
	Softer	0	0	
Hair present* before sammying and staking	Less	2	11	
	Equal	17	15	
	More	5	0	
Temper* after burnishing	Firmer	23	25	
	Equal	1	1	
Break* after burnishing	Looser	3	10	
	Equal	17	15	
	Tighter	4	1	
Grain smoothness* after burnishing	Coarser	22	20	
	Equal	2	5	
	Smoother	0	1	

*Test sides compared with the controls. The figures refer to the number of occurrences in the test sides.

TABLE I (Continued)

Characteristics		Test	Control	Test	Control
<i>Finished Leather†</i>					
<i>Temper*</i>	Firmer	22		21	
	Equal	0		2	
	Softer	1		3	
<i>Break*</i>	Tighter	0		7	
	Equal	18		3	
	Looser	5		16	
<i>Grain smoothness*</i>	Coarser	17		21	
	Equal	3		4	
	Smother	3		1	
<i>Veins*</i>	More	16		19	
	Equal	7		7	
<i>Measurement, sq. ft., avg.</i>		19.9	19.4	20.3	19.85
<i>Selection</i>	No. 1	0	0	0	0
	2	0	5	0	3
	3	3	9	0	8
	4	10	8	9	11
	5	9	1	17	4
	Reject	1	0	0	0

*Test sides compared with the controls. The figures refer to the number of occurrences in the test sides.
†One side lost in the first run.

The firmness of the enzyme-unhaired sides persisted in the finished leather, as did the looser break and the coarser grain. The leather from enzyme-unhaired sides also had more prominent veins than that from the lime-sulfide-unhaired sides. The use of caustic in the soak may have accentuated these conditions.

On the basis of the sorters' very extensive examination and evaluation, the leathers from the enzyme-unhaired sides were downgraded because of the looser break, coarser grain, prominent veins, and firmer temper. However, this firmer temper may be desirable in some types of shoe construction.

In Table II the arithmetical averages and the analysis of variance of the data on the physical properties of the leather are given. The leathers from the enzyme-unhaired hides had significantly higher tensile strength, grain crack, and compressibility. The higher values of the first two of these properties are advantageous, but the third is directly related to the firmer temper. The leather from the lime-sulfide-unhaired hides had significantly greater elongation at break and stitch tear than the test leather. However, the stitch tear values of the experimental leather were still within the acceptable range.

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TABLE II
PHYSICAL PROPERTIES OF SIDE LEATHER

	First Run		Second Run	
	(Averages, 24 sides)		(Averages, 26 sides)	
	Enzyme	Lime	Enzyme	Lime
Tensile strength, p.s.i.	3240*	2973	2447†	2373
Elongation at break, %	21.97	31.58‡	24.04	33.44‡
Mullen grain crack, lb.	611†	522	471‡	387
Stitch tear, lb/in	769	1095†	885	1008†
Compressometer reading (temper)	78‡	49	59‡	36

Analysis of variance:

*90% probability level

†95% probability level

‡99% probability level

The chemical analyses of the side leathers are given in Table III. The results show that there is little difference in chemical composition of the leathers from the two unhairing systems. The leathers from the second run contain about 2% more grease than those from the first run. This probably accounts for the lower compressometer readings (softer temper) of the leather from the second run.

TABLE III
CHEMICAL ANALYSES OF SIDE LEATHERS FROM
ENZYME-UNHAIRING SIDES VERSUS LIME-SULFIDE-UNHAIRING SIDES

Chemical Composition	First Run		Second Run	
	Enzyme Stock	Lime Stock	Enzyme Stock	Lime Stock
Moisture	12.62	12.63	14.33	14.36
Grease (M.F.B.)	5.62	5.13	7.46	7.28
Hide substance (M.F.B.)	78.04	78.18	76.15	75.94
Total ash (M.F.B.)	6.65	6.23	6.28	6.10
Chrome (M.F.B.)	5.09	4.76	5.24	5.43
pH	3.51	3.52	3.35	3.49

Shrinkage temperature of leathers was the same, both lots standing the boil. There was no difference in the deposition of the chrome as shown by incineration of micro cross sections.

Microscopic examination of cross sections of the leathers showed no difference in fiber bundle pattern. However, it was observed that the dye had penetrated into the grain side of the leather prepared from the lime-sulfide-unhaired hides, but it remained practically on the grain surface of the leather prepared from enzyme-unhaired hides. This retention of the dye on the surface gave a much glossier leather than the regular leather. This characteristic was not noted by the tannery sorters in their evaluation of the leathers.

Conclusions.—Results of these tests show that enzyme-unhaired stock can be handled with regular production equipment and integrated into the regular pickle-tan, fatliquoring, coloring, and finishing operations. No loss in area or thickness is experienced during production. On the basis of physical tests, chemical analyses, and observations made on cross sections, there is little difference in the leather from enzyme-unhaired stock compared with lime-sulfide-unhaired stock. However, the firmness, roughness of grain, and veininess resulted in a downgrading of the leather from the enzyme-unhaired hides. More work needs to be done to overcome these defects.

Sole Leather

Materials and procedures

Ten runs of five hides each were made.

Hides.—Heavy hides were cut down the backbone, and matched sides were used for enzyme unhairing tests and lime-sulfide-unhaired controls.

Soak.—Test stock was washed 10–15 min., then soaked overnight in water at 60°–65°F. This was followed by a 23-hr. soak in 0.125% sodium hydroxide solution at a 4:1 float. The pH of the soak water was 11.9–12.0 at the start and 10.5–11.2 at the finish.

Wash.—A 5-min. wash in a drum with slatted door followed by a 15-min. still soak was repeated three times. The pH of the final wash was 7.9–8.9.

Enzyme treatment.—To hides in the drum was added a solution containing

M-ZYME®	190 K units per ml.
Sodium chloride	5%
Borax	0.7%
Water	400% on the hide weight

The pH at start was 8.5–8.8, and at the finish it was 8.2–8.6. The temperature was set at 89°–92°F., and at the finish it was 80°–88°F.

After 24 hr. in the unhairing solution the sides were put through the regular unhairing, fleshing, and graining operations. They were then washed for 5 min. in a revolving drum and allowed to stand 15 min. covered with water. This washing was repeated two additional times, after which the sides were hung in the rockers with the regular tannery stock. The remaining operations were those used for the regular production of sole leather.

Evaluation of leather.—Samples of sole leather were analyzed at several periods during tanning: after 7–9 days in the rockers, out of the rockers, after the layaway, after tempering, and after finishing. Determinations were made for grease, hide substance, water solubles, insoluble ash, and combined tannin (9). The physical properties determined were tensile strength, elongation at break, stitch tear, abrasion resistance, compression, and density (9). Tannery personnel determined finished weights and yields of bends, bellies, and shoulders and also evaluated the leather for thickness, color, and general appearance. Cross sections of the finished leather were examined microscopically for fiber bundle pattern, fat deposition, and general structure. (11).

Results and discussion—Examination of Table IV shows that there is little difference in the thickness of the bend leather from the two unhairing treatments. Twenty of the test bends had lighter but less uniform color than the control bends. Eleven of these bends showing uneven color were in the last three runs, and these bends all had some fine hair indicating that possibly a longer time in the unhairing bath may correct this condition at this tannery.

TABLE IV
TANNERS' EVALUATION OF SOLE LEATHER BENDS

Thickness Irons	Totals	Tannery Sort			Off Color	
		Better	Standard or Acceptable	Special or Reject	Better	Standard or Acceptable
<i>Test bends</i>						
8–9	2			2		
9–10½	43	2	8	16	4	13
10½–13	10		2	5		3
Totals	55	2	10	23	4	16
<i>Control bends</i>						
8–9	2		2			
9–10½	38	8	18	12		
10½–13	14	4	5	2	1	2
Totals	54	12	25	14	1	2

It is significant that with three trial runs at another tannery this condition was not encountered. The data given in Table V show that there was no significant difference in the average weights of the stock used in each of the comparative tests. Enzyme-unhaired stock gave slightly better yields of leather, and in the case of the shoulders the difference was significant at the 95% level of probability. The over-all yield from the enzyme-unhaired stock was 73.4%; from the lime-sulfide-unhaired stock it was 68.7%.

TABLE V
SOLE LEATHER YIELDS AND WEIGHTS

	Averages, lb.	
Stock weight	163.4	162.5
Bends	62.2*	59.5
Bellies	27.0†	25.5
Shoulders	30.7‡	26.6

Analysis of Variance:

*92% probability level

†91% probability level

‡95% probability level

The results of the physical tests on the sole leather bends from the ten runs of five hides each are given in Table VI. Analysis of variance showed that the probability of the leather from the enzyme-unhaired stock being stronger was 91%. The probability figures for elongation at break and stitch tear were 95% and 92% respectively in favor of the control bends. There was no significant difference between the leathers with respect to abrasion resistance, compressibility, and density. The lack of difference in compressibility, as shown by the compressometer, does not bear out the experience of the tannery personnel who cut samples for the physical tests. They thought the leather from the enzyme-unhaired hides was a little more difficult to cut, indicating a more compact fiber structure.

None of the leathers showed any piping when bent grain in, or cracking when bent grain out, over a 3-inch mandrel.

In Table VII the average chemical composition is given of 10 sides after 7-9 days in the rocker section, out of the rocker section, and after tempering, and of 7 sides after layaway. The sides were analyzed individually. Examination and statistical analysis of the data show that there was very little difference in the behavior of the test and control stock during tannage. There was no significant difference in the degree of tannage at any of the stages in the production process where samplings for analyses were made. Tannin had

TABLE VI
PHYSICAL PROPERTIES OF SOLE LEATHER

	Averages	
	Test	Control
Tensile strength, p.s.i.	2695*	2515
Elongation at break, %	11.86	13.05‡
Stitch tear, lb/in	312	368†
Abrasion, mils/rev	0.61	0.60
Compression, %	3.88	3.99
Density, gm/cm ³	0.972	0.970

Analysis of Variance:
 *91% probability level
 †92% probability level
 ‡95% probability level

completely penetrated the stock at the time it was through the rocker section except for the bend area of a few hides, and no raw streaks were found when the bends were sampled after layaway and after tempering.

Five matched half-sides were sampled in the "A" position and analyzed individually, and the data were analyzed statistically. The results are given in Table VIII. The only instances of significant differences were a slightly higher insoluble ash in the limed stock, which is certainly of no consequence, and a higher degree of tannage of the enzyme-unhaired stock. Table VIII also contains the results of the chemical analysis of composite samples from 43 half-sides each sampled in the A, B, and J positions. These results agree very well with those obtained by the analysis of individual hides. The chemical composition of the finished leather from the two unhairing treatments is remarkably uniform. Furthermore, there was no difference in fiber bundle pattern, general structure, or fat deposition as shown by microscopic examination of cross sections.

Conclusions.—Based on physical test results, chemical analyses, and examination of cross sections there is little difference in the sole leather produced from enzyme-unhaired half-sides compared with the lime-sulfide-unhaired mates. It is possible to produce sole leather from enzyme-unhaired stock using the regular tannery equipment and tan yard without any changes in the tanning liquors. Area, thickness, and yield of leather were essentially the same for the two unhairing systems. Some of the bends unhaired with enzyme had drawn grain, uneven color, and fine hair. A 48-hour period in the enzyme bath should eliminate the fine hair as it did at the side leather tannery and might also improve the uneven color. Additional work should be carried out to correct these defects.

TABLE VII
CHEMICAL ANALYSES OF THE STOCK IN THE SOLE
LEATHER TAN YARD FROM ENZYME-UNHAIRIED SIDES
VERSUS LIME-SULFIDE-UNHAIRIED SIDES

	7-9 Day Rocker		Out of Rocker		After Layaway		After Temper	
	10 Sides Each		10 Sides Each		7 Sides Each		10 Sides Each	
	Enzyme	Lime	Enzyme	Lime	Enzyme	Lime	Enzyme	Lime
Grease, %	3.62	4.14	3.11	3.24	2.70	2.68	3.34	2.95
Hide substance, %	68.02	66.49	49.50	48.97	46.30	45.97	46.67	46.05
Water solubles, %	9.27	10.65*	15.97	16.42	16.06	16.36	14.03	15.01
Insoluble ash, %	0.25	0.30†	0.19	0.19	0.19	0.18	0.20	0.21
Combined tannin, %	18.84	18.42	31.23	31.18	34.75	34.81	35.76	35.78
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Soluble nontannin, %	5.40	5.19	5.42	5.17	3.41	3.61	2.63	2.87
Uncombined tannin, %	4.77	5.46‡	10.56	11.25**	12.66	12.75	11.40	12.14
Total ash, %	1.35	1.44	0.97	0.92	0.55	0.50	0.56	0.62
pH (Acidity)	4.78	4.66	3.62	3.57	3.42	3.39	3.54	3.54
Moisture, %	10.89	11.17	8.59	9.10	9.83	9.94	9.65	10.10
Degree of tannage	27.87	27.84	63.18	63.69	75.05	75.78	76.68	77.73

Individual analysis run on a side from each of ten runs and averaged except for stock out of layaway where only sides from seven runs were sampled for analysis for each run.

Analysis of Variance:

*90% probability level
†91% probability level
‡95% probability level
**88% probability level

TABLE VIII
CHEMICAL ANALYSES OF FINISHED LEATHER FROM
ENZYME-UNHAIRED VERSUS LIME-SULFIDE-UNHAIRED HALF-SIDES

	Finished Stock		Finished Stock	
	5 Sides Each "A" Position*		43 Sides Each A,B,J Positions**	
	Test	Control	Test	Control
Grease, %	5.30	5.12	4.25	5.18
Hide substance, %	35.71	36.51	35.14	34.56
Water solubles, %	30.19	30.35	33.65	33.48
Insoluble ash, %	.15	.16	.16	.18
Combined tannin, %	28.65	27.86	26.80	26.60
Total	100.00	100.00	100.00	100.00
Soluble nontannin, %	20.30	21.47	22.39	21.10
Uncombined tannin, %	9.89	8.88	10.79	12.38
Total ash, %	6.39	5.98	6.40	6.80
pH (Acidity)	3.26	3.23	3.28	3.28
Moisture, %	11.47	11.32	11.04	10.82
Degree of tannage	80.20†	76.39	76.25	76.97

*Individual analysis run on a specimen from the A position from a side from each of five runs and averaged.

**Pieces from the A,B,J positions from the bend area of 43 sides from each treatment composited for chemical analysis.

†Analysis of variance: 95% probability level.

SUMMARY

It has been shown that enzyme-unhaired hides can be converted into side upper leather or sole leather using standard tannery equipment. Only minor changes are required in the processing for side leather production, and the same tan yard process can be used for sole leather production as is used in the regular procedures. There are still minor defects in both types of leather when made from enzyme-unhaired hides using unmodified conventional tanning methods, and more studies will be required to adjust existing processes to correct these deficiencies.

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REFERENCES

1. Greene, G. H. *J. Soc. Leather Trades Chemists*, **36**, 127 (1952).
2. Merrill, H. B. in the *Chemistry and Technology of Leather*, ed. O'Flaherty, F., Roddy, W. T., and Lollar, R. M. (New York: Reinhold Publishing Corp., 1956), Chap. 8.
3. Hollander, Chas. S. *JALCA*, **15**, 477-486 (1920).
4. Cordon, T. C., Windus, W., Clark, I. D., and Naghski, J. *JALCA*, **54**, 122 (1959).
5. Everett, A., and Cordon, T. C. *JALCA*, **53**, 548 (1958).
6. Cordon, T. C., Jones, H. W., Clark, I. D., and Naghski, J. *Appl. Microbiology*, **6**, 293 (1958).
7. Grimm, O. *Das Leder*, **10**, 244 (1959).
8. Lindroth, E. G. Thesis, University of Cincinnati (1959).
9. ALCA Methods of Sampling and Analysis. Available from the Secretary-Treasurer, Dr. Fred O'Flaherty, Tanners' Council Research Laboratory, University of Cincinnati, Cincinnati 21, Ohio.
10. Mattei, V., and Roddy, W. T. *JALCA*, **54**, 12-27 (1959).
11. Tancous, J. J., Roddy, W. T., and O'Flaherty, F. *Skin, Hide and Leather Defects*. (Western Hills Publishing Co., 1959; distributed by the Tanners' Council Laboratory.)

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